

REMARKS

Claims 1-68 are pending. The Examiner withdrew claims 51-67 from consideration. Claims 1-50 and 68 are under consideration.

Applicants have amended claims 10 and 35 to change the language "a group comprising" to the language "at least one of." Applicants have amended claims 11, 12, 36, and 37 to add the language "at least" before the word "one." Applicants have amended claims 13 and 38 to replace the word "or" with the word "and." These amendments do not narrow the scope of the claims. Applicants have also amended claims 16 and 41 to add an "s" to the word "indicate." No new matter is added by these amendments.

The following remarks address the Examiner's comments in the Advisory Action mailed September 24, 2003.

I. Rejection of Claims 10 and 35 Under 35 U.S.C. § 112, 2nd paragraph

The Examiner maintained the rejection of claims 10 and 35 under 35 U.S.C. § 112, second paragraph, alleging that "it is still unclear whether or not the nucleic acid from the virus or prokaryote as listed in the claims is chemically modified." (Advisory Action at page 2.) Responding to the Applicants' assertion that the nucleic acid may or may not be chemically modified, the Examiner alleged that "the phrase [sic, phrase] 'derived from' in the art . . . describes a chemical compound which has a chemical modification." (*Id.*) This rejection is respectfully traversed.

The Examiner presents no evidentiary support for her assertion that "derived from" is a term of art that describes a chemical compound that has a chemical modification. Applicants disagree that the term "derived from" requires a chemical

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modification. Applicants request that the Examiner clarify the basis in the art for asserting that "derived from" requires the presence of a chemical modification.

Furthermore, the specification uses the term "derived from" in a manner consistent with its plain meaning. For example, the specification states that "[t]he target polynucleotide may be *derived from* any biological source, including, but not limited to, viruses, prokaryotes, protists, eukaryotes, plants, fungi, and animals." (Specification at page 7 (emphasis added).) That disclosure refers to the biological source without limiting the target polynucleotide to either a chemically modified or unmodified form. Therefore, the Examiner should interpret "derived from" accordingly. See M.P.E.P. § 2111.01 at 2100-47 (8th ed. rev. 1, Feb. 2003) ("During examination, the claims must be interpreted as broadly as their terms reasonably allow. This means that the words of the claim must be given their plain meaning . . .").

The Examiner has provided no legal basis for requiring that the claims recite *either* chemically modified nucleic acids *or* unmodified nucleic acids. The claims as written encompass *both* chemically modified *and* unmodified nucleic acids. Therefore, the rejection of claims 10 and 35 under 35 U.S.C. § 112, second paragraph, is improper. Withdrawal of this rejection is respectfully requested.

II. Rejection of claims 26-50 under 35 U.S.C. § 112, 2nd paragraph

The Examiner maintained the rejection of claims 26-50 under 35 U.S.C. § 112, second paragraph, alleging that "it is still unclear how the sequence of the at least one amplification product of the first reaction composition is determined since the first

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reaction composition does not have a fluorescence indicator.”¹ (Advisory Action at page

2.) Specifically, the Examiner stated that:

it is unclear how to determine whether the at least one amplification product is present in both the first reaction composition and the second reaction composition from the intensity of signal from the fluorescent indicator in the second reaction composition. [In other] words, there is no a [sic] relation between the first composition and second composition.

(Advisory Action at page 3.) This rejection is respectfully traversed.

The relationship between the first and second reaction compositions is clearly set forth in the claims. Nucleic acid from the same sample is combined with the first and second reaction compositions. Both reaction compositions are “specific for the at least one target polynucleotide,” and both comprise “amplification primers specific to the at least one target polynucleotide.” Thus, one can determine whether the at least one amplification product is present in the first reaction composition based on the intensity of signal from the fluorescent indicator in the second reaction composition, because of the above relationship between the first and second reaction compositions.

Applicants respectfully request withdrawal of the rejection of claims 26 to 50 under 35 U.S.C. § 112, second paragraph.

III. Rejection of Claims 1-25 and 68 Under 35 U.S.C. § 103(a)

The Examiner maintained the rejection of claims 1-25 and 68 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Pritham et al. (J. Clinical Ligand Assay (1998) 4:404-412), in view of Johnston-Dow et al. (U.S. Patent No.

¹ In the response filed August 11, 2003, at page 3, Applicants noted that techniques for sequencing were known that did not invoke fluorescent indicators. Determining the sequence according to claims 26 to 50 may or may not invoke fluorescent indicators, as discussed in the response filed December 5, 2002, at pages 6-7.

6,103,465). Because the Examiner has not established a *prima facie* case of obviousness, this rejection is respectfully traversed.

No Motivation To Combine

To establish a *prima facie* case of obviousness, the Examiner must provide evidence of motivation to combine the cited documents. Motivation must come from *outside Applicant's disclosure*, i.e., in the cited documents or from the knowledge generally available to one of ordinary skill in the art. See M.P.E.P. § 2143.01 at 2100-125; see also *In re Oetiker*, 24 USPQ2d 1443, 1446 (Fed. Cir. 1992) ("[Motivation] can not come from the applicant's invention itself."). In discussing motivation, the Examiner stated the following:

in the last step of claim 1 it states "determining the sequence of the at least one amplification product if the at least one amplification product is present" without any specified steps for sequencing. *Thus as long as any method which is used for sequencing, it would have been used for "determining the sequence of the at least one amplification product if the at least one amplification product is present"*Because of the benefit of using the DNA sequencing of Johnson-Dow et al., *one of ordinary skill in the art would have been motivated to apply the sequencing method of Johnson-Dow et al. to determine the sequence of the at least one amplification product if the at least one amplification product is present.*

(Advisory Action at pages 3-4 (emphasis added).) The Examiner is essentially arguing that one skilled in the art, *having knowledge of Applicants' claimed invention*, might choose the sequencing method of Johnston-Dow to achieve a particular element of the claimed invention. This argument, however, does not supply the requisite motivation to combine Johnston-Dow with Pritham. "The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination." See M.P.E.P. § 2143.01 at 2100-126 (citing *In re Mills*, 16 USPQ2d 1430 (Fed. Cir. 1990)).

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Furthermore, this argument is clearly based on improper hindsight reconstruction. “One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention.” *In re Fine*, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988); see also *In re Dembicza*k, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999) (“Combining prior art references without evidence of such a suggestion, teaching, or motivation simply takes the inventor’s disclosure as a blueprint for piecing together the prior art to defeat patentability – the essence of hindsight.”). Here, the Examiner simply chose Johnston-Dow to accomplish the “determining the sequence” claim element, without providing any motivation to combine Johnston-Dow with Pritham. This is particularly apparent in view of the Examiner’s statement that “any method which is used for sequencing . . . would have been used for ‘determining the sequence of the at least one amplification product . . .’” (Advisory Action at page 3.) Therefore, the Examiner has failed to show that one skilled in the art, lacking any hindsight knowledge of Applicants’ claimed invention, would have been motivated to combine Johnston-Dow with Pritham.

No reasonable expectation of success

Not only is there no motivation to combine the cited documents, there is also no reasonable expectation of success. Claim 1 recites “. . . determining the sequence of the at least one amplification product if the at least one amplification product is present.” The sequencing of amplification products was not predictable at the time the invention was made, as shown by Sambrook et al., *Molecular Cloning: A Laboratory Manual* (3rd ed. 2001) (enclosed). For example, Sambrook states that:

Methods to sequence the amplified double-stranded products of PCRs are attractive in theory but unreliable in practice. . . . However, direct sequencing of

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double-stranded PCR products remains a technical challenge, as the diversity and number of papers on the topic make manifest. . . . The keys to success are rigorous optimization of the amplification step to suppress mispriming and meticulous purification to rid the PCR product of residual primers, thermostable DNA polymerase, unused dNTPs, and nonspecific reproductions of the original template.

(See page 12.106, first and second paragraphs.) Therefore, there was no reasonable expectation of success in “determining the sequence of the at least one amplification product if the at least one amplification product is present.”

Because the Examiner has shown neither motivation to combine Pritham and Johnston-Dow nor a reasonable expectation of success, the Examiner has failed to establish a *prima facie* case of obviousness. Therefore, Applicants respectfully request withdrawal of the rejection of claims 1-25 and 68 under 35 U.S.C. § 103(a).

IV. Rejection of Claims 26-50 Under 35 U.S.C. § 103(a)

The Examiner maintained the rejection of claims 26-50 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Pritham and Johnston-Dow, in further view of Wittwer et al. (U.S. Patent No. 6,174, 670). This rejection is respectfully traversed.

The Examiner alleges that “the motivation of applying the teachings of Wittwer et al. is that the method of Wittwer et al. improves the sensitivity of PCR quantification and reduces the time of fluorescence monitoring for PCR.” (Final Office Action at page 6.) This statement merely recites certain general features of the method of Wittwer. It does not explain the motivation to combine Wittwer with the other cited documents. See *In re Dembiczaik*, 50 USPQ2d at 1617, 1618 (stating that “evidence of a suggestion, teaching, or motivation to combine . . . must be clear and particular,” and further remarking that the Board of Patent Appeals and Interferences failed to point out “specific information in [the cited documents] that suggest the combination. . . .”).

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Furthermore, Wittwer fails to teach or suggest all the elements of claims 26-50. Claim 26 recites “at least one set of reaction compositions comprising a first reaction composition and second reaction composition” Wittwer discusses a method that comprises a single reaction mixture. (See col. 13, line 65, and col. 14, line 14, which the Examiner cites in the Final Office Action at page 5.) Wittwer does not teach or suggest “at least one set of reaction compositions comprising a first reaction composition and second reaction composition” Therefore, Wittwer, in combination with Pritham and Johnston-Dow, fails to teach or suggest all the elements of claim 26 and claims 27-50, which ultimately depend from claim 26.

Applicants therefore request reconsideration and withdrawal of the rejection of claims 26-50 under 35 U.S.C. § 103(a).

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CONCLUSION

Applicants respectfully request the reconsideration and reexamination of this application and the timely allowance of the pending claims. In the event that the Examiner does not find the claims allowable, Applicants request that the Examiner contact the undersigned at (650) 849-6778 to set up an interview.

Please grant any extensions of time required to enter this response and charge any additional required fees to Deposit Account No. 06-0916.

Respectfully submitted,

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Dated: February 11, 2004

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VOLUME 2

Molecular Cloning

A LABORATORY MANUAL

THIRD EDITION

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Front cover (paperback): The gene encoding green fluorescent protein was cloned from *Aequorea victoria*, a jellyfish found in abundance in Puget Sound, Washington State. This picture of a 50-mm medusa was taken on color film by flash photography and shows light reflected from various morphological features of the animal. The small bright roundish blobs in the photograph are symbiotic amphipods living on or in the medusa. The bright ragged area in the center is the jellyfish's mouth.

Bioluminescence from *Aequorea* is emitted only from the margins of the medusae and cannot be seen in this image. Bioluminescence of *Aequorea*, as in most species of jellyfish, does not look like a soft overall glow, but occurs only at the rim of the bell and, given the right viewing conditions, would appear as a string of nearly microscopic fusiform green lights. The primary luminescence produced by *Aequorea* is actually bluish in color and is emitted by the protein aequorin. In a living jellyfish, light is emitted via the coupled green fluorescent protein, which causes the luminescence to appear green to the observer.

The figure and legend were kindly provided by Claudia Mills of the University of Washington, Friday Harbor. For further information, please see Mills, C.E. 1999–2000. Bioluminescence of *Aequorea*, a hydromedusa. Electronic Internet document available at <http://faculty.washington.edu/cemills/Aequorea.html>. Published by the author, web page established June 1999, last updated 23 August 2000.

Back cover (paperback): A portion of a human cDNA array hybridized with a red fluor-tagged experimental sample and a green fluor-tagged reference sample. Please see Appendix 10 for details. (Image provided by Vivek Mittal and Michael Wigler, Cold Spring Harbor Laboratory.)

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CONVENTIONAL CHAIN-TERMINATION SEQUENCING OF PCR-AMPLIFIED DNA

Methods to sequence the amplified double-stranded products of PCRs are attractive in theory but unreliable in practice. The methods appeal to the investigator who wants to sequence either or both strands of template DNA, and they offer the ability to generate templates for sequencing without the labor of cloning into bacterial or bacteriophage vectors. However, direct sequencing of double-stranded PCR products remains a technical challenge, as the diversity and number of papers on the topic make manifest.

The keys to success are rigorous optimization of the amplification step to suppress mispriming and meticulous purification to rid the PCR product of residual primers, thermostable DNA polymerase, unused dNTPs, and nonspecific reproductions of the original template. Purification generally involves chromatography through spun columns or commercial resins, followed by elution of the desired fragment from an agarose or polyacrylamide gel. With this amount of work, it is perhaps not surprising that many investigators turn to cycle sequencing (please see Protocol 6) or opt for the well-trodden traditional route of cloning PCR products into plasmids or bacteriophage M13 vectors.

Because of differences in the size, yield, and specificity of the amplification product, none of the available purification methods can be used to purify each and every PCR product. However, with some effort, it is almost always possible to devise a successful purification scheme for the products of a specific PCR. The following guide lists the main options that are available. Detailed protocols for many of these options are presented in other sections of this manual.

- ***Removal of Residual Primers and Unused dNTPs***

Spun column chromatography through Sepharose CL-6B or Sephadryl S-400 (please see Appendix 8). Sepharose CL-6B equilibrated in TE (pH 7.6) excludes double-stranded DNAs >190 bp in size. Sephadryl S-400 excludes double-stranded DNAs >260 bp in size. Both matrices will retard the passage of dNTPs, primers, and primer-dimers.

Centrifugal ultrafiltration through Centricon-100 or Microcon-100 units (Krowczynska and Henderson 1992; Leonard et al. 1998; please see Chapter 8, Protocol 3).

- ***Elimination of Residual Thermostable DNA Polymerase***

Taq polymerase, and presumably other thermostable DNA polymerases, survive extraction with phenol:chloroform, ethanol precipitation, and other regimens commonly used to purify the products of PCR (Crowe et al. 1991; Barnes 1992). The continuing presence of the DNA polymerase together with residual dNTPs may befoul chain-termination sequencing reactions catalyzed by other thermostable enzymes such as Thermo Sequenase and AmpliTaq. A method to remove residual thermostable DNA polymerase is described in Chapter 8, Protocol 3.

- ***Elimination of Nonspecific Amplification Products***

Target DNA can be separated from amplified DNAs that differ in size by electrophoresis through low melting/gelling temperature agarose (please see Chapter 5). The desired PCR product can then be recovered from the gel by one of the methods described in Chapter 5.

When the PCR yields a heterogeneous set of products of similar size, it may be necessary to carry out a second round of PCR using nested primers, or to use a restriction enzyme to cleave unwanted products, or to use a more sophisticated electrophoretic system, for example, denaturing gradient gel electrophoresis (Fischer and Lerman 1983; for review, please see Myers et al. 1998).

Finally, some of the difficulties encountered in sequencing amplified DNAs are not unique. Like any other double-stranded DNA, amplified DNAs will inevitably reassociate after denaturation, whereupon the sequencing primer may be unable to anneal or the DNA polymerase may be unable to proceed along the full length of the template (Gyllensten and Erlich 1988). These problems can be minimized by rapidly transferring the amplified DNA, after denaturation by heat (Kusukawa et al. 1990) or alkali (Wrischnik et al. 1987), to conditions that do not favor reassociation of DNA strands, namely, low temperature and low ionic strength. The quicker the denatured DNA is used in the annealing and extension/termination reactions, the better (please see Protocol 2).